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(54) Title: PROCESS FOR SEPARATING LIPIDS AND PROTEINS FROM BIOLOGICAL MATERIAL (57) Abstract <p>A process for separating non-denatured fat and lipids as well as non-denatured proteins from a material of organic origin is disclosed wherein the fat and proteins are separated at a temperature below the denaturing temperature for the proteins to conserve their biological function and nutritional value.</p>		

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Process for separating lipids and proteins
from biological material.

The present invention relates to a process for separating
5 and producing a material being of an organic biological
origin and containing lipids and proteins, especially
separating and producing such a material from aquatic
organisms, e.g. fish. The present invention is especially
concerned with producing oil and lipids from vegetables
10 and/or fish and/or other animals wherein the oil, lipids
and/or fat contains a larger amount of non-degraded
proteins and other essential materials (vitamins, bio-bound
nutritional, poly-unsaturated fats etc).

15 It is previously known methods for producing and separating
different materials from living organisms. Among these it
may be mentioned the production of fat and oils from fish,
mammals and plants, the production of proteins and
proteinaceous material from animals and plants, the
20 production of dyes, vitamins etc. from animal and plant
materials, etc.

Among the techniques being closest to the present invention
is the process being disclosed in NO patent application no.
25 1993 3009 wherein it is disclosed a method for producing
oils with a high oxidative stability from marine raw
materials. In this previously known process for the raw
material is frozen rapidly with a freezing rate of >
1°C/min. and is processed in a frozen condition, whereafter
30 the fat is separated with a gentle heating and by the fat
being melted from the raw material. In this patent it is
disclosed how the freezing of the raw material is done to
conserve the oil quality being wanted in the end product,
and what parameters are active in such a process. However,
35 not much is indicated concerning the indicated slow heating
of the material for melting the fat and oils from the
starting material.

It is previously also known similar methods for producing oil, e.g. through NO patent 43.956 and 38.027. Thus it is known from NO patent to freeze fish liver and thaw this in a per se known manner followed by a separation of the oil for producing fish oil. Likewise it is known from NO patent 38.027 to freeze fish liver and thaw this in a per se known manner followed by separation of the oil for producing fish oil. Likewise it is known from NO patent 38.027 to freeze fish liver to rupture the liver cells and thus make the fish oil, after thawing, separable from the other material. In none of these patents it is indicated anything about how the thawing is to be performed and neither anything about the effects that the thawing process will have on the thawed material.

The present invention is an inventive development of the methods being disclosed in the above indicated patents/patent applications and which the present invention also may use for producing and separating material which is of a biological origin and which contains lipids and proteins. The purpose of such a separation is to produce an oil with a content of fat/oil (lipids) together with proteins being present in their natural condition, i.e. not being denatured. The purpose of this is that the fat/oil fraction in different biological materials contains substances being of a great nutritional and medical importance, and it is consequently of great consequence to avoid denaturation of such components when separating fat/oil from the biological material. This is relevant for animal material (marine and non-marine) as well as vegetable material, and among vegetable material it may in this connection be mentioned separation of oil from soy, olives, sunflower, corn, etc.

Since separation of oil from marine material in one of the separation forms which lies closest to the present invention, effects of heating in traditional separation of marine oils will be considered.

The current methods for separating cod liver oil comprise heating cod liver by using water steam to the extent that the cell structure is damaged and the proteins are
5 denatured. This heating of the raw material leads to the cod liver oil, being non-miscible with water, floating to the surface and it is then separated from the aqueous material by centrifuging, pressing, floatation of decanting.

10 Fish oil is produced as a bi-product in the production process of fish meal. The quality of the oil reflects this as well. The process for producing oil starts with wet-pressing of the raw material. The raw material is treated
15 with heat by heating this to between 70-90°C, and this denatures the proteins being present in the material. However, the fat/oil is liberated in this process. The fat/oil may then be separated by the methods indicated supra. It has been possible to reduce the time that the
20 raw material is subjected to this heating, and it has thus been possible to improve the quality of the product, but nevertheless it has been necessary with such a steam treatment to separate out the wanted lipids.

25 In addition to the proteins becoming denatured by the heat treatment, other components such as thermal labile anti-oxidants, pro-oxidants, enzymes and fatty acids of longer chains and/or with a large degree of non-saturation become partly or completely destroyed in such a heat process as
30 indicated supra. In addition the supply of large amounts of energy brings about activation and high reaction rates in the material such as auto-oxidation, photo-oxidation, enzymatic oxidation and polymerization.

35 Heat treatment causes also activation of oxidation reactions either purely thermal or through the reduction of the content of thermolabile anti-oxidants. A too strong heating has also the result that natural substances

contributing to making the material stable towards becoming rancid, are inactivated. By heating the material, and thereby denaturing the proteins, metal ions will also be liberated. Examples are ferritin and haemoglobin which
5 when denatured, liberate iron. Ions of transition metals, e.g. iron (Fe^{3+}) and copper (Cu^{2+}) are examples of strong pro-oxidants contributing to a faster oxidation of the fat and thus a poorer stability.

10 Techniques avoiding heating the material to any substantial degree, have also been used for the separation of oil and fat. This is especially true for so-called "cold-pressing" at about 30-50°C of vegetable oils from vegetable material after a mechanical treatment of the material, but here the
15 material has not previously been subjected to a freezing process. "Cold pressing" has also be done when separating cod liver oil, but here the fat often is, in addition to the moderate heating, is subjected to temperatures about 90°C to make the process more effective in a subsequent
20 separation step.

The goal for the present invention is to produce a lipid/proteinaceous product with as little denaturing as possible of the product's components, where the process
25 contributes with the stabilisation of the product, i.e. exposes it to as little oxidative stress as possible, and at the same time avoids adding air (oxygen) and liberating pro-oxidants such as metal ions, and wherein the temperature may be kept low (under the denaturing
30 temperature for the proteins in the biological material) and where the process may be performed in darkness to avoid photo-oxygenation of the material. Additionally it may be possible to perform the process according to the present invention under an inert atmosphere (e.g. under nitrogen or
35 argon) or in a vacuum to avoid oxidation of the lipids, proteins or trace elements in the treated biological material.

The heating of the material by the process according to the invention may be done with any conventional heating apparatus suitable for avoiding any substantial denaturing of the treated material during the heating process. As
5 examples of such heating processes it may be mentioned heat exchanging, microwave heating, infra red light heating, electric heating or any other mild heating process.

The process according to the invention has as its starting
10 point a material of an organic biological origin (fish liver, whale blubber, soy beans, olives, sunflower seed, corn, microorganic material (yeast, bacteria, cell culture material) etc.) containing lipids and proteins. An
optional starting material is such a material as indicated
15 supra having been pre-treated to weaken or rupture to cell walls. Such a treatment may be done with enzymes (e.g. cellulase, collagenase, lysozym etc.) and/or with surfactants and/or with solvents (e.g. hexane) and/or with emulsion-bursting compounds or compositions (e.g. salt
20 solutions) and/or emulsion-inhibiting solutions etc. Such treatment may be used to improve the yield of cellular materials or components (fat and/or proteins) by rupturing the cell wall to liberate the cell components such as the fat.

25 This material is frozen to a low temperature. The freezing temperature is not critical, but it lies in such an interval that the membranes surrounding the cells in the frozen material become "brittle" and rupture to liberate
30 the contents of the cells. Normally it will be sufficient to freeze the material to a temperature within the interval 0°C to -10°C, more preferred 0°C to -6°C, even if other temperature intervals are possible, e.g. temperatures from -3°C to -50°C, preferably -5°C to -28°C. The freezing ratio
35 (speed) may be relatively rapid, preferably over 1°C/min, even if this is neither of any significant importance for the process according to the present invention.

Without being limited by theory, it is presumed that the consequence of freezing is focused about a repacking of the membrane lipids. For the depot fat in biological material, which mainly comprises triglycerides, the freezing has no significant consequences, other than the volume in the fat vacuoles shrinking with about 10%. For the membrane lipids, however, the repacking by freezing may directly damage the membrane structure. In membranes, which in their original form has a liquid heterogenous structure of phospholipids, the freezing gives rise to a phase separation where the phospholipids are crystallised out as separate "islands" in the membrane. This phase separation is not reversible and the changes affect the membrane's function after thawing. In a frozen condition water, fat and tissue (cells) will form a solid, rigid structure. This rigid structure is ice crystals, cell membrane and fat vacuoles is exploited in a mechanical preparation of the frozen tissue. The elasticity of the cell membrane in a frozen condition is gone, and it is not possible for the tissue in a frozen condition to distribute forces being supplied thereto. Supplied external forces will thus be sufficient to crush the tissue so that the oil may be extracted by a moderate heating. In a rapid freezing, the used freezing time for the tissue will, as mentioned supra, advantageously be cut, and the lipids and proteins will be exposed to the fewest possible attacks of oxidative substances being present in the material.

Additionally, in a slow freezing fewer cores, and thus larger ice crystals, will be formed. After a while the salt concentration in the cells will become so high that this in itself has pro-oxidative properties. An increased enzyme activity is shown in the temperature interval for the forming of ice (-2°C to 0°C). This is also a reason for it being preferred that the freezing process for the biological material is made as small as possible to obtain stable oils of high quality from the biological material.

According to the present invention the raw material is not subjected to temperature conditions wherein unwanted taste and colour substances are dissolved in the fatty phase or which destroy the natural anti-oxidants. This is achieved
5 by the raw material first being frozen rapidly so that the water in the material forms many small ice crystals and the cell membranes and other cellular components are kept in a solid, rigid structure. After freezing the raw material is treated mechanically, e.g. by cutting, milling, 10 scraping, pounding or in any other way. This increases the surface area of the frozen material so that a separation of the lipids, after a moderate heating, may be separated from the rest of the biological material. It is preferred, but not necessary, that the mean particle diameter after the 15 cutting/grinding does not exceed 50 mm, and preferably does not exceed 25 mm.

The thawing of the material may proceed either by thawing the material in an oven or by supplying microwaves or in 20 any other conventional way. The requirement is that the thawing does not denature the proteins in the lipid phase.

As a special consideration the origin of the relevant organic material will have to be taken into account.

25

Proteins from e.g. cold water fish species have been shown to denature at as low a temperature as 25°C. The normal physiological functional temperature for these species is about 10-15°C or less. This means that the fish have their 30 proteins and the melting temperature of their fat especially adapted to this temperature. Fish from temperate waters have a considerably higher denaturing temperature of their corresponding substances.

35 This means that for any kind of organic material one has to find where the denaturing starts to obtain the best possible effect of separation with the process according to the present invention.

Viscosity measurements of ground, whole frozen liver from cod shows that a denaturing starts already at about 40°C. The same result was found for the proteinaceous phase separated by the process according to the present invention. The viscosity decreased from 10°C to 40°C for the proteinaceous phase and increased thereafter to above 80°C. The oil per se decreases in viscosity from 10°C and up to 50°C and above.

Accordingly it is important for the efficiency to have as high a temperature as possible without harming the functionality of the proteins. This depends on which function or use of the proteins that is wanted.

At a temperature of over 30°C it becomes difficult to separate the liver mass and this is on account of the nature of the material.

What happens in the liver mass may actually be observed. The structure of the proteins is held together partly by "sulphur bridges" and partly by hydrogen bonds (Van der Waals forces) forming across the proteins' structure from protein "strand" to protein "strand" and also to other types of cellular components. The consequences of the heating is that when heat energy is supplied, the motional energy (rotational and translatory energy) of the molecules increases. When the energy of motion surpasses the Van der Waals forces, the original structure of the proteins will change and start to move and rearrange itself. The proteins will now start to bunch and stick to surfaces and partly form new hydrogen bonds at other and completely random locations. This makes the separation difficult. The fat will in a way become emulsified by the long collecting arms of the proteins in this phase.

In cod liver it is about 25-30% water and only 6-8% protein and about 60-70% oil. When the temperature increases

slightly more water becomes separated from the protein. The emulsifying action increases and it becomes even harder to perform a separation.

- 5 When using the process according to the present invention it is accordingly important to avoid that three phases are formed, i.e. a proteinaceous phase, free water and oil. A three phase separator is only able to handle 2 liquid phases and in a case where water is formed freely there
10 will rapidly be formed an emulsion.

When the temperature reaches the interval 55-95°C, the proteins start to agglomerate to small protein clusters. Water may only bind in small quantities to these protein
15 clusters which have formed. This causes the surface area and the density of the proteins to increase and the particle diameter to increase. Simultaneously the difference in specific weight between water and oil is
20 lower temperatures, and this makes a further separation easier to perform, something which may account for the fact that most prior art separation is performed at elevated temperatures. This is illustrated in the figure given
25 infra (Figure 1). This figure depicts the graphs of the specific gravity of water versus the temperature and the specific gravity of cod liver oil versus the temperature in the temperature interval 0-95°C

Specific gravity versus temperatur

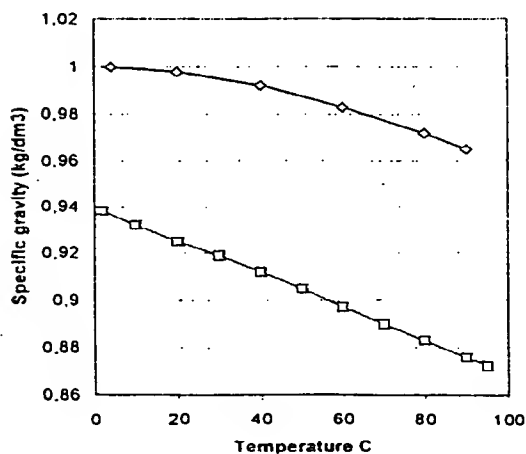


Figure 1

5

What is lost at such high temperatures are important thermolabile components such as retinol (vitamin A) and other important trace elements such as natural anti
10 oxidants etc. The functional properties of the proteins are lost through denaturing.

On the other hand the proteins from the protein phase obtained by the process according to the present invention,
15 may be concentrated and isolated, and they may be used as functional proteins in the nutritional industry and the pet food industry to bind fat and have also other functional tasks.

20 The oil from the process according to the present invention may, when it is refined and deodorized, or when it is in some other way treated so that its taste becomes neutral, be used as an additive in nutritional substances (food and/or feed) to give such substances a better nutritional
25 value, and it may thereby represent a substantial improvement for the industrial user. To improve the stability of the oil and/or the proteins, antioxidants may also be added in the raw material and/or in the refined material.

30

Likewise the quality of the oil produced according to the present invention is an excellent starting point for using the oil in other areas, e.g. by using the oil as a raw material in enzymatic esterification (concentration, inter-
35 esterification, construction of novel triglycerides with a defined composition of the fatty acids ("structured lipids")). With a low degree of oxidation the lipids produced according to the present invention represents a

unique basic material, and the oil thus obtained may accordingly withstand a greater degree of handling and processing without this having large consequences for the quality of the end product.

5

Thus the oil *per se* produced by the process according to the present invention, also represents an aspect of the invention.

10 The process according to the invention is characterised by the steps, being performed subsequently to the freezing and the division and diminution of the pieces of biological material, of thawing the frozen and processed material and
15 isolating the oil at a temperature where the proteins do not start to denature, said denaturing temperature being determined by a visual inspection of the oil where the maximum temperature is the temperature where proteins in the oil start to form agglomerates visible as strings or a precipitate in the oil.

20

Said maximum temperature is specific for the relevant organisms from which the oil is separated. As mentioned
supra organisms from cold environments will normally have a lower maximum isolation temperature for the oil than
25 organisms from a more temperate habitat. However, merely easy and simple ordinary tests obvious for the person skilled in the art are necessary to determine said maximum temperature. Examples of this is given *infra*.

30 As a preferred and optimum process, however, is a modified process to the one indicated *supra*. The modified process is one where there is taken account of the fact that the oil to be extracted from the biological material becomes more fluid the more elevated the temperature. Accordingly,
35 there exists an optimal temperature for any given biological material where the proteins still are in their natural and undenatured state, but where the oil is as

fluid as possible for cold extraction according to the process indicated supra.

When thawing the frozen and processed material there will
5 exist a particular temperature above which the proteins in the material start to denature, as disclosed supra. This will normally be observed by the structure of the material changing from a composition where fat and proteins are kept in a structural mesh or array which normally exists in such
10 a material, to pass over to a more liquid phase, whereafter the structure, at a given temperature dependent on the relevant species or organism from which the material is taken, quickly passes from being ordered to become more random since the structuring and holding mesh or array in
15 the material becomes broken down when the proteins start to denature on account of the heat and on account of the increasing denaturing reactions occurring at elevated temperatures (see supra). It is presumed that this temperature, wherein fat/proteins are not exposed to any
20 significant denaturing, lies within the interval 0 - 60°C, but it will be an easy matter for the person skilled in the art to determine the specific denaturing temperature for the relevant biological material both by observation (see supra) and by optionally measuring the viscosity of the
25 relevant raw material and/or of the fatty phase. When separating fat and protein from the rest of the biological material in the process according to the invention, it is required that the lipids at the relevant temperature (below the denaturing temperature for the proteins) is in a liquid
30 state so that non-denatured proteins and fat/oil may be separated from the material in a per se conventional manner.

When determining the optimum temperature for extracting the
35 oil according to the inventive process, if such an extraction is performed by using a two-phase or three-phase separator, the heat provided by the separator will have to

be included in the estimation of the highest temperature to use for the relevant biological material.

5 Since the extraction of the oil according to the invention is performed at a temperature below the denaturing temperature of the proteins of the material, a subsequent step may be performed for isolating the relevant proteins from the oil. Examples of such proteins (or other essential nutritional or trace materials) are given supra,
10 and it may especially be mentioned protein extracts from soy beans or other sources as interesting materials to be isolated by the process according to the invention.

15 As an alternative it may also be possible to perform the diminution/mechanical processing of the biological material prior to the freezing of the material.

Fat with a high degree of unsaturation has a low melting point, and the process according to the present invention
20 is accordingly especially suited for isolating/extracting raw material which is rich in this kind of fat/oil. Specific examples of such biological materials are cod liver, soy beans, sunflower seed, olive seed, as well as algae and other microorganisms.

25 In a traditional extraction of fat from raw materials as indicated supra, there is commonly used high temperatures in the raw material for extracting the fat, and the remaining material (the grax) will comprise *inter alia*
30 denatured proteins, and under this denatured binding tissue. This grax has, however, a poor nutritional value as a source for proteins on account of a poor digestibility. An alternate method for extracting fat at low temperatures, and especially one where the main part of
35 the proteins are not denatured, gives a grax with a far better nutritional value. At such a low temperature process the proteins are not denatured and the oxidation of the lipids is restricted or inhibited. Such a grax will

accordingly be a good source for different proteins, and the grax produced according to the present invention may thus be used as a nutritional substance *per se*, or may be used further as a raw material for isolation of proteins or
5 trace elements which did not pass into the fatty phase.

In an optional and preferred process it is possible to separate the freezing and thawing sections of the biological starting material so that the freezing and
10 thawing processes may be performed continuously.

The invention will infra be disclosed through simple and non-limiting examples.

15 Example 1

500 kg cod liver was frozen in a plate freezer to a core temperature of -22°C. The liver was then crushed in a mill and ground to a particle size of about 0,5 cm. The liver was then treated in a pump and further transported in a
20 heat exchanger where the frozen material was thawed to a temperature of 28°C and then pumped into a three phase separator.

Oil was isolated via the light phase and the protein mass
25 was isolated via the heavy liquid phase exiting the separator. The yield was measured to be 52% w/w of the oil and the rest was isolated as a proteinaceous phase.

Example 2

30 Cod liver (3.500 kg) was frozen like in Example 1 and treated similarly up to the heating sequence. In this example the liver material was thawed to 10°C and then pumped to the separator. The capacity was then determined to obtain a useful separation. Subsequently the
35 temperature was increased to 15°C, 20°C, 25°C, 30°C and up to 40°C. The yield of oil was recorded at representative temperatures during the heating process to obtain the optimum temperature at which to separate the relevant

phases. As another item to take into consideration is also the capacity of the separator to be used in the process. Since the process according to the invention is performed at relatively low temperatures, the speed of the separator will have to be reduced to obtain the improved product according to the invention. The optimal speed versus temperature for the relevant separator within the separation parameters according to the invention may also be determined by performing a similar test.

The following results were obtained:

Table 1

Temperature	Result (kg/h)
10	150
20	400
25	500
28	600
31	Sep. problems, poor yield
40	No separation

Example 3

The proteinaceous phase from Example 1 was examined for its functional valuable properties. A representative sample of about 7 kg was removed from the relevant phase. The sample was homogenised with water in a 1 : 1 ratio and the pH was adjusted to pH 9 and sentrifuged to concentrate the proteinaceous phase. The water-soluble proteins were removed in the supernatant from the sentrifuging (hereafter called the protein extract).

The non-water soluble pellet was discarded.

A sample of about 7 kg from the proteinaceous phase from Example 1 was also taken out and lyophilised (infra called lyophilised protein).

- 5 The functional properties from each of the relevant materials (the protein extract and the lyophilised protein) were examined and compared with two different samples of commercial casein (EM7 and EM65). The results are given in table 2.

10

Emulsifying properties:

Table 2

Sample	Emulsifying activity	Emulsifying stability (%)
Protein extract	0,408	64
Lyophilised protein	0,335	57
EM7 (comm. casein)	0,336	69
EM65 (comm. casein)	0,378	60

15

The fat-binding properties of the material is given in Table 3 infra:

Table 3:

20

Sample	Fat-binding property (g fat/g protein)
Lyophilised protein	4,5
Protein extract	4,4
EM7/EM65 (comm. casein)	2,0

The gel-forming properties of the material is given in Table 4 infra:

25 Table 4:

Sample	Viscosity prior to heating	Viscosity after heating and cooling
Lyophilised protein (10%)	Thin paste .	Firm gel
Protein extract (10%)	Thin paste	Firm gel
Lyophilised protein (5%)	Liquid dispersion	Firm gel
Protein extract (5%)	Liquid dispersion	Intermediate between thick paste and a gel
Protein phase (raw)	Liquid dispersion	Firm gel

Example 4

3500 kg cod liver was frozen to -11°C, treated in a mill, pumped through a heat exchanger and thawed to a temperature of 27°C. The liver material was then separated as in Example 1, and the oil phase was removed and measured for quality. The elements in the oil are indicated in Table 5 infra.

10 Table 5:

Analysis	Result
Free fatty acids (%)	09
Peroxide number (meq/kg)	1,0
Anisidine value	0,6

Example 5

The oil from Example 4 was subsequently refined, deodorised and added a commercial antioxidant (tocopherol-mixture). The elements in the oil after this treatment is given in Table 6 infra.

20 Table 6:

Analysis	Result
Free fatty acids (5)	0,06
Peroxide number (meq/kg)	0,4
Anisidine value	0,6
Taste and smell	Neutral

Subsequently the oil was mixed in a liquid condition into a nutritional substance such as bread (0,6%) and margarine (5%).

5

All of the applications created taste-neutral products which in a sensoric panel were impossible to distinguish from the reference samples.

10 Example 7

Frozen cod liver (2 kg) was milled and heated in intervals, and was sentrifuged in a laboratory scale sentrifuge. The yield of the oil phase was measured and the content of vitamin A was measured in the oil. The yield of the oil in % versus the temperature is given in figure 2 infra. The figure shows that the yield passes through a minimum at a temperature of about 35-45°C. The corresponding measured data for the yield as well as the data for the content of vitamin A in the oil are given in Tables 7 and 8 infra.

15
20Table 7:

Yield of the oil versus temperature

Temp.	10	20	30	40	50	60	70	80	90
Yield oil (% w/w)	62	63	45	34	54	58	60	63	65

25 Table 8:

Content of vitamin A found in the isolated oil.

Temp °C	20	40	50	65	80	95
Vit. A (µg/g)	529	522	518	513	494	402

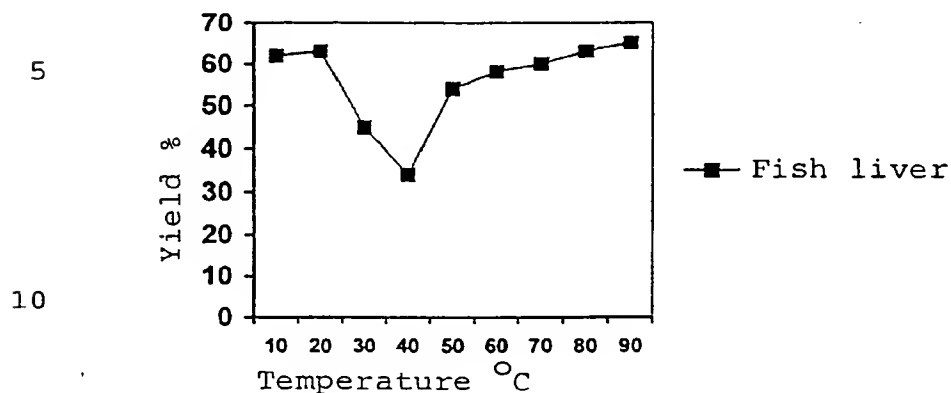


Figure 2

Example 8

Cod liver material was processed as in Example 1. On material from a separation on an industrial scale, there was performed tests on frozen ground liver, separated oil and the protein phase from the separator concerning the viscosity as a function of the time and the temperature. The results are given in the figure infra and in table 9 infra.

Temperature °C	Cod Liver Protein Viscosity cp	Cod Liver Viscosity cp	Temperature °C	Cod Liver Oil Viscosity cp
10	510	2000	0	228
15	450	1700	10	108
20	390	890	20	69
30	260	600	30	56
40	165	520	40	46
50	203	600	50	38
60	335	560		
70	575	500		
80	1000	200		

Table 9

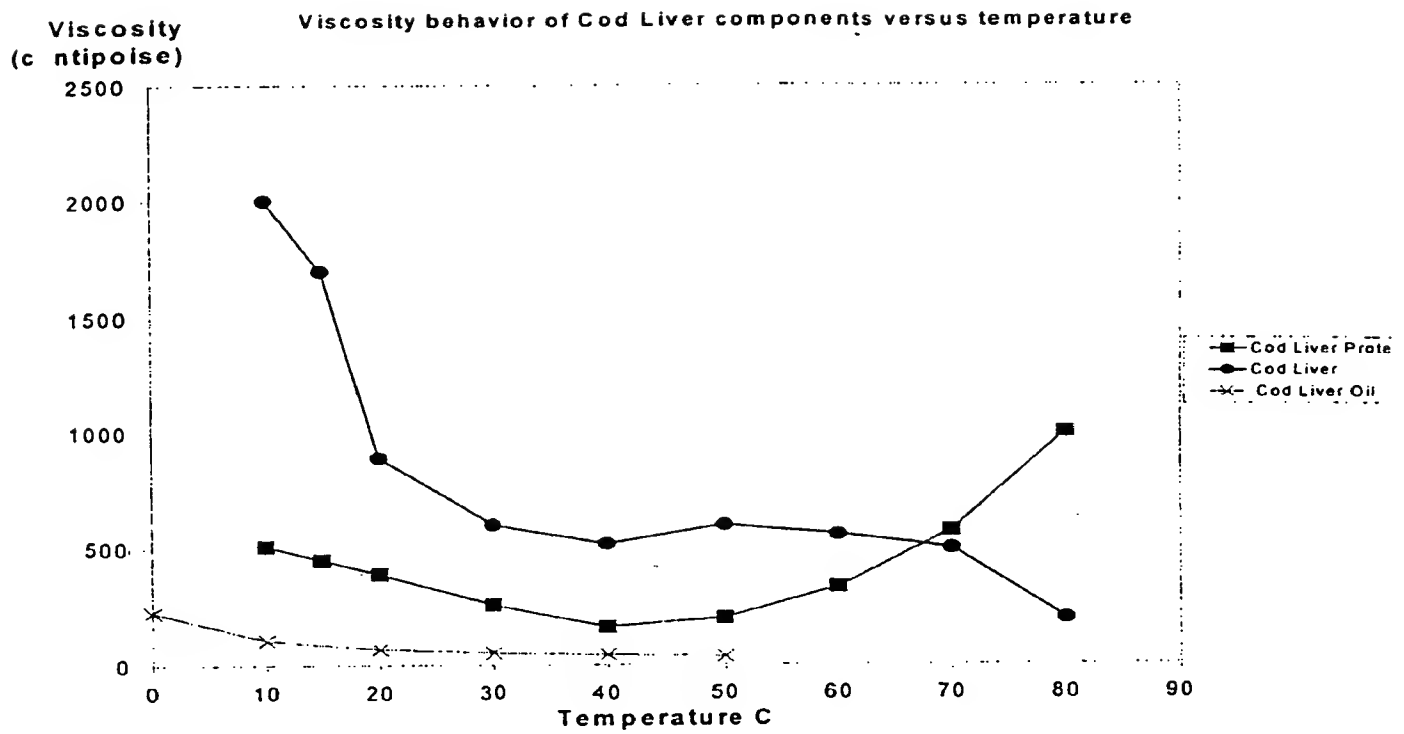


Figure 3

C l a i m s

1. Process for separating and isolating nutritional
elements from a material containing lipids and proteins
and being of a biological origin, in which process the
biological material is either frozen and cut into
smaller pieces or vice versa, the material being
mechanically processed while frozen to rupture the cell
membranes in the biological material and thus destroy
structural components in the material,
c h a r a c t e r i z e d i n that the biological
material subsequently is thawed to a temperature within
the interval 0°C to 60°C, although below the denaturing
temperature for the proteins, sad denaturing
temperature being the temperature where the proteins in
the material start to form agglomerates being visible
as strings or a precipitation in the material,
whereafter protein and fat/lipids are separated from
the resulting composition at a temperature below the
found denaturing temperature in a per se known manner,
the material being processed at a temperature in the
interval 0°C to 60°C provided the lipids under these
conditions are liquid and where the proteins are not
denatured during the separation.

2. Process according to claim 1,
c h a r a c t e r i z e d i n that the freezing/thawing
processes are performed continuously.

3. Process according to claim 1,
c h a r a c t e r i z e d i n that the freezing/thawing
process is performed semi-continuously.

4. Process according to any of the claims 1 - 3,
c h a r a c t e r i z e d i n that the material is
frozen to a temperature within the interval -3°C to -50°C,
preferably within the interval -5°C to -28°C.

5. Process according to any of the claims 1 - 4,
c h a r a c t e r i z e d i n that the mechanical
processing of the biological material is performed by one
or more of the methods grinding, milling, chopping or
5 pressing.
6. Process according to any of the preceding claims,
c h a r a c t e r i z e d i n that a non-denatured oil
including non-denatured proteins is isolated from the
10 biological material.
7. Process according to any of the preceding claims,
c h a r a c t e r i z e d i n that the grax being left
over after the processing of the biological material is
15 used as a nutritional additive in food or feed, or that the
grax serves as a starting material for further isolation of
non-denatured proteins or non-denatured trace elements.
8. Process according to claim 7,
20 c h a r a c t e r i z e d i n that the isolated trace
elements are vitamins.
9. Process according to any of the preceding claims,
c h a r a c t e r i z e d i n that the process is
25 performed under a vacuum or under an inert atmosphere.
10. Process according to any of the preceding claims,
c h a r a c t e r i z e d i n that cell disrupting
compounds or compositions are added to the raw material
30 prior or subsequent to the diminution of the raw material.
11. Process according to any of the preceding claims,
c h a r a c t e r i z e d i n that the cell disrupting
compound(s) is/are enzyme(s), solvent(s), emulsion-bursting
35 material(s), emulsion-inhibiting solution(s).
12. Process according to any of the preceding claims,

c h a r a c t e r i z e d i n t h a t o n e o r m o r e a n t i -
o x i d a n t (s) i s / a r e a d d e d d u r i n g t h e p r o c e s s .

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 99/00321

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C11B 1/00, C11B 1/02, C11B 1/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C11B, A23D, A23J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NO 9303009 A (NORSK INSTITUT FOR FISKERI- OG HAVBRUKSFORSKNING), 29 December 1994 (29.12.94) --	1-12
A	Pauling, L., College Chemistry, Third Edition, W.H. Freeman and Company, page 732 "The Denaturation of Proteins" - page 733, line 12 -- -----	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

2 February 2000

Date of mailing of the international search report

17 -02- 2000

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INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.

PCT/NO 99/00321

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
NO 9303009 A	29/12/94	NONE	